This microscopic understanding paves the way for the rational design of protein mutants able to selectively target subcellular compartments thanks to altered membrane binding properties.

1908-Plat

In Situ Synthesis of Fluorescent Membrane Lipids (Ceramides) using Click Chemistry

L. Ruth Montes[1], Maria Garrido[2], Jose Luis Abad[2], Antonio Delgado[2,3], Felix M. Goni[1], **Alicia Alonso**[1].
[1]Unidad de Biofisica (CSIC-UPV/EHU), Leioa, Spain, [2]Research Unit on Bioactive Molecules (RUBAM), Institut de Quimica Avançada de Catalunya (IQAC-CSIC), Barcelona, Spain, [3]Facultad de Farmacia, Unidad de Quimica Farmaceutica , Universidad de Barcelona, Barcelona, Spain.

Ceramide analogues containing azide groups either in the polar head or in the hydrocarbon chains are nonfluorescent. When incorporated into phospholipid bilayers, they can react in situ with a non-fluorescent 1,8-naphthalimide using click chemistry giving rise to fluorescent ceramide derivatives emitting at $\approx$440 nm. When incorporated into giant unilamellar vesicles, two-photon excitation at 760 nm allows visualization of the ceramide-containing bilayers. We have also proven that this technique is also suitable for its use in cell systems. When incubated with HEK 293 cell lines we have seen internalization and redistribution of our molecules within the cell. We have also seen what seems to be accumulation of ceramides in a speckle pattern. This kind of method may be of general applicability in the study of model and cell membranes.

1909-Plat

Orientation of Proteorhodopsin in Artificial Membranes is affected by Lipid Bilayer Composition

Ramya Tunuguntla[1], Mangesh Bangar[2], Kyunghoon Kim[3], Pieter Stroeve[1], Caroline Ajo-Franklin[2], Aleksandr Noy[4].
[1]UC Davis, Fremont, CA, USA, [2]Lawrence Berkeley National Lab, Berkeley, CA, USA, [3]UC Berkeley, Berkeley, CA, USA, [4]Lawrence Livermore National Laboratory, Livermore, CA, USA.

In artificial membrane systems we want to study the structure and function of proteins as they would behave in their natural environment, so they can be used in applications that require an ex vivo approach. For instance, proteins are increasingly used in FET biosensors, thin-film protein arrays, or bio-fuel cells. These applications require a controlled protein orientation after immobilization onto varying surfaces. When two orientations are present in a system, the average functionality may not always be sufficient to acquire a signal. However, an anisotropic orientation can lead to homogeneity in transport direction and therefore increase signal response. Our objective was to determine whether the surface charge on a liposome plays a key role in determining transmembrane protein orientation and functionality during formation of proteoliposomes. To study this, we reconstitute a model ion pump, proteorhodopsin, in liposomes of opposite charges and varying charge densities and observe the resultant protein orientation. We used four different assays to study the electrostatic protein-surface interactions and light-driven ion transport in proteoliposomes. The surface treatments and proteolysis of proteoliposomes showed physical evidence of preferential orientation, while functional assays provided evidence of vectorial ion transport. We show that the manipulation of lipid composition can control orientation of protein in liposomes. This technique opens up a simple route for controlling protein orientation in many applications ranging from solution vesicle assays to complex bioelectronic devices and sensors that use membrane proteins.

1910-Plat

Molecular pH Probes at a Protein-Lipid Interface: Assessment of Local Dielectric Environment for Transmembrane Peptide

Matthew Donohue, Maxim A. Voynov, Alex I. Smirnov,
Tatyana I. Smirnova.
North Carolina State University, Raleigh, NC, USA.

Spin-labeling EPR spectroscopy has found many applications in studying structure and dynamics of proteins and biological membranes. Recently, there has been substantial interest in utilizing EPR to characterize local effects of polarity and hydrogen bonding in these systems. Here we report on employing an pH-sensitive EPR probe IMSTL (S-(1-oxyl-2,2,3,5,5-pentamethylimidazolidin-4-ylmethyl) ester) to profile heterogeneous dielectric environments along the α-helix of a WALP peptide integrated in a lipid bilayer. A series of symmetrically positioned double cysteine mutants were labeled with a pH-sensitive nitroxide and the protonation state of IMTSL was directly observed by EPR. Q-band DEER experiments with double-labeled WALPs were employed to derive nitroxide-nitroxide distances of nitroxides before and after the protonation and, therefore, the positions of pH probes with respect to lipid bialyer. Thus, for the first time measurements of local electrostatics at peptide-bilayer interface were based on direct distance measurements rather than on assumptions on the probe location. For double-labeled WALP consecutive protonation of symmetrically positioned nitroxide tags was observed. The difference in observable pKa values was interpreted in terms of electrostatic interaction energy between titratable probes allowing us to estimate effective dielectric constant. Supported by NSF-0843632 to TIS.

1911-Plat

A New Assay for Ion Channel Function using Stopped Flow Spectrofluorometry

Radda Rusinova, Dorothy Kim, Crina Nimigean, Olaf S. Andersen.
Weill Medical College of Cornell University, New York, NY, USA.

Functional studies of purified ion channels reconstituted in a defined membrane environment usually are conducted using single-channel electrophysiology. Ensemble-averaged methods are used infrequently, compared to single-channel electrophysiology because of the technical challenges involved in giant unilamellar liposome preparation and patching—though these methods yield important information that may be difficult to obtain from single-channel studies, e.g. (Chakrapani et al., 2007). To overcome some of the limitations encountered, we developed a sequential-push fluorescence-based stopped-flow assay to characterize channel function, using KcsA as the prototype. The method is based on earlier studies (Moore and Raftery 1980; Wu et al., 1981; Ingólfsson and Andersen), where channel activity was determined using the KcsA-permeable ANTS quencher, thallium (Tl^+). We evaluate KcsA function from the rate of ANTS quenching in sequential-push stopped-flow measurements on the millisecond timescale. Detergent was removed using BioBeads, which minimizes ANTS consumption. To illustrate the method, we determined the KcsA activation by extravesicular H^+ for WT KcsA and the non-inactivating E71A mutant channels. Our results demonstrate efficient and reproducible reconstitution. Fitting the activation curve using the Hill equation yields a pH for half-activation ($pH_{0.5}$) similar to that obtained from single-channel electrophysiology for the E71A mutant (Thompson et al., 2008) and higher than that reported for WT KcsA (Chakrapani et al.). The Hill coefficients, n, were lower than those obtained with single-channel recording for E71A mutant but higher for WT KcsA. Our results suggest that both $pH_{0.5}$ and n vary with changes in lipid bilayer thickness and bulk properties such as elasticity. The ease of vesicle preparation, cost effectiveness, reproducibility and time resolution makes this a powerful assay to explore KcsA activation and inactivation, and how they vary with changes in the channels' lipid bilayer environment.

1912-Plat

Study of Nanoscopic Phase Separation in Membranes using Inverse FCS

Yanfei Jiang[1], Guy Genin[2], Elliot L. Elson[1].
[1]Washington Univ Sch Med, St Louis, MO, USA, [2]Washington University School of Engineering, St Louis, MO, USA.

Fluorescence correlation spectroscopy (FCS) has been used to study the diffusion of lipid and protein molecules in both synthetic and natural membranes. Recently, a new version of FCS, inverse FCS, has been developed (Wennmalm et al., 2009). For inverse FCS fluorescent probes are present at very high concentration as opposed to low concentration for conventional FCS. The particle of interest in the inverse FCS experiment is non-fluorescent. Fluorescence fluctuations due to the exclusion of fluorescent probes from the excitation spot by the non-fluorescent particles are detected and correlated. In the study of membrane nanodomains by conventional FCS, both domains and probe diffusion contribute to the correlation fluctuation. However, for inverse FCS, the diffusion of fluorescent probes contributes little to the fluctuation due to the high concentration of fluorescent probes. Therefore, inverse FCS provides an opportunity to study the properties of dark nanodomains that exclude the fluorescent probe when the fraction of nanodomains in the membrane is small. Here, we demonstrate by simulation that in these situations inverse FCS can give information about membrane nanoscopic phase separation that is inaccessible to conventional FCS. We also used inverse FCS to experimentally study early phase separation in DLPC/DSPC model membranes. Two different domain evolution pathways have been observed. In one of these two pathways, nanoscopic domains appear at first and then gradually grow to micron size. In the other pathway, the domains reach micron size quickly and their number gradually increases.

Inverse-Fluorescence Correlation Spectroscopy, Stefan Wennmalm, Per Thyberg, Lei Xu, and Jerker Widengren. Anal. Chem. 2009, 81, 9209-9215

1913-Plat

A Revised Model of Nonyl Acridine Orange Binding to Anionic Phospholipids

Piercen M. Oliver, John A. Crooks, Earl J. Yoon, Douglas B. Weibel.
UW-Madison, Madison, WI, USA.

This presentation describes mechanistic studies of the interaction of nonyl acridine orange (NAO) with anionic phospholipids (PLs). NAO has become a widely used reagent for quantifying, labeling, and visualizing the unique